

Detection of Nitrobenzene in Biological Materials by Thin Layer Chromatography

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Abstract

A thin layer chromatographic method is developed for the detection of nitrobenzene in biological material. Nitrobenzene on reduction gives aniline, a primary amine which on diazotization and coupling with β -naphthol, gives orange coloured μ spot (i.e. aniline dye). The detection limit for nitrobenzene on TLC plate in biological material is 5 μ g. The biological impurities such as amino acids, fats etc. present in visceral material do not interfere in the test. The method has been successfully employed for the detection in forensic samples.

Key Words: Nitrobenzene, Thin layer chromatography, Diazotization, β -naphthol

INTRODUCTION

Generally Nitrobenzene is widely used in the manufacturing of aniline, in soap, shoe polishes for refining lubricating oils¹. Now a days a new formulation containing 20% v/v solution of Nitrobenzene is introduced which act as a plant energizer, flowering stimulant and yield booster in agriculture. Nitrobenzene is volatile; it has a boiling point of 211°C and a vapor pressure (20°C) of 0.15 mmHg. Its water solubility (20°C) is 1,900 mg/L. It has a log(octanol/water) partition coefficient value of 1.85, implying a relatively weak affinity for lipids. These properties affect the manner in which biological samples are analyzed for nitrobenzene.

Nitrobenzene can cause a wide variety of harmful health effects to exposed persons. Direct contact of small amounts of nitrobenzene with the skin or eyes may cause mild irritation. Repeated exposures to a high concentration of nitrobenzene can result in a blood condition called methemoglobinemia. This condition affects the ability of the blood to carry oxygen. Following such an exposure, the skin may turn a bluish color. This may be accompanied by nausea, vomiting and shortness of breath. Effects such as headache, irritability, dizziness, weakness, and drowsiness may also occur. If the exposure level is extremely high, nitrobenzene can cause coma and possibly death unless prompt medical treatment is received. Consuming alcoholic beverages during nitrobenzene exposure may increase the harmful effects of nitrobenzene.

In studies with laboratory animals, a single dose of nitrobenzene fed to male rats resulted in damage to the testicles and decreased levels of sperm. This suggests that decreased fertility may be a concern in humans. There is very little information available about the effects of long-term exposure of humans or animals to nitrobenzene, and it is not known whether exposure to nitrobenzene can cause cancer. Albrecht and Neumann³ discussed the difficulty of analysis of nitrobenzene and its metabolite aniline in animals. Excretion of the parent compounds or some metabolites in urine has been determined, but apparently this kind of biological monitoring has so far not produced satisfactory results due to practical and methodological reasons. Nitrobenzene metabolites are bound to blood proteins, both in hemoglobin and in plasma. Acute poisoning by nitrobenzene is usually monitored by measuring levels of methemoglobin, which is produced by the metabolic products of nitrobenzene. However, many toxicants produce methemoglobin, and this analysis is not specific enough to be a satisfactory method for monitoring nitrobenzene in animals.

However, it is misused for suicidal / homicidal purposes. In such poisoning cases the autopsy surgeon preserves the postmortem samples for chemical analysis and sends to the Forensic Science Laboratories. Several methods such as ultraviolet spectrophotometry⁴, gas chromatography and high performance liquid chromatography⁵ have been reported in the literature for the detection of nitrobenzene but these methods require elaborate instrumental assay. Thin-layer chromatography (TLC) has a long history, but has been used only to a limited extent in pesticide residue analytical laboratories since gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) became readily available. Recent developments in the quality of plate coating and detection systems, as well as in extraction and clean up method have revived interest in TLC. Nitrobenzene has been determined in environmental samples (air, water, soil and solid waste samples) by GC analysis following collection and extraction with an organic solvent⁶⁻⁸; flame ionization or mass spectrometry (MS) may be used for detection. Lewalter & Ellrich⁹ reported a capillary GC method for nitroaromatic compounds, including nitrobenzene, in plasma samples. No TLC method found reported in the literature for its detection in the biological material.

During routine screening usually occurring poisons i.e. organochloro, organophosphorus, carbamate, pyrethroids etc by Thin layer chromatographic method there are chances of getting skip out the detection of nitrobenzene. Hence a study was undertaken to detect nitrobenzene by thin layer chromatographic method, where nitrobenzene was successfully reduced to amine on TLC plate using stannous chloride which on diazotization and coupling with β -naphthol gives an orange colored spot. This can be routinely used in such Forensic biological samples. The sensitivity of the reagent was about $\sim 5 \mu\text{g}$.

Experimental

Chemicals & Reagents

All chemicals & reagents used were of AR grade. (Benzene, Acetone, Absolute Alcohol, Hydrochloric acid, Sodium Nitrite, Stannous chloride, β -Naphthol) & distilled water was used throughout. Extraction from viscera: - 100 gms of minced postmortem sample (stomach, intestine etc.) was taken, approximately 10 gm of ammonium sulphate was added and was extracted with diethyl ether. The extracted volume was evaporated at room temperature. The residue was dissolved in absolute alcohol for spotting.

Standard Solution

a) A 100 mg % solution of Technical grade Nitrobenzene from SD Fine Chemical Limited, Mumbai (10mg/ 8.3 ml) was prepared in absolute alcohol.

b) Spray reagent:-i) Aqueous solution of 10% v/v Hydrochloric Acid, ii) Aqueous solution of 2% w/v Sodium Nitrite, iii) 5% w/v solution of stannous chloride in cone. HCl, iv) 10% β -Naphthol in 10% w/v Sodium hydroxide v/ere prepared.

Thin-layer chromatography Conditions

TLC Plate: A standard glass TLC plate was coated with 0.25mm layer of silica gel -G slurry in distilled water. The plate was dried at room temperature and activated at about 100°C for 1 hour in an oven.

Spotting device: An aliquot of 10 μ L volume of extracted material and standard Nitrobenzene solution was spotted on to the TLC plate. Then 0.1 mL of 5% w/v stannous chloride solution was spotted on to the same location. Then the plate was heated at 100°C for 10 minutes to complete reduction. The plate was cooled to room temperature.

Development Chamber: It was then developed to a distance of 10 cm in presaturated TLC chamber using benzene: acetone: ethyl alcohol (8:1:1) as a mobile phase. After development, the plate was dried in air and uniformly sprayed with aqueous solution of 2% w/v sodium nitrite followed by 10% alkaline β -naphthol. An intense orange colored spot was visualized immediately at $R_f \times 100 = 25$. The colour of spot remains stable for couple of days.

Results and discussion

Nitrobenzene Fig. 1 is reduced to aniline, which is a primary aromatic amine readily undergoes diazotization in the presence of stannous chloride, the diazonium salt is coupled with at 1 - position of (β -naphthol to give an orange colored azo dye. The colour of the spot is stable for couple of days. The solvent system used gives compact spot. The biological impurities such as amino acids, fats etc. present in the visceral material do not interfere in the test. Other insecticides such as carbamates (Baygon, Carbaryl & Carbofuran) do not interfere in this test as they are not hydrolysed to corresponding phenols in the acidic condition¹⁰.

The proposed reagent is more sensitive for the detection of Nitrobenzene in biological samples. (Viscera and gastric aspirate).

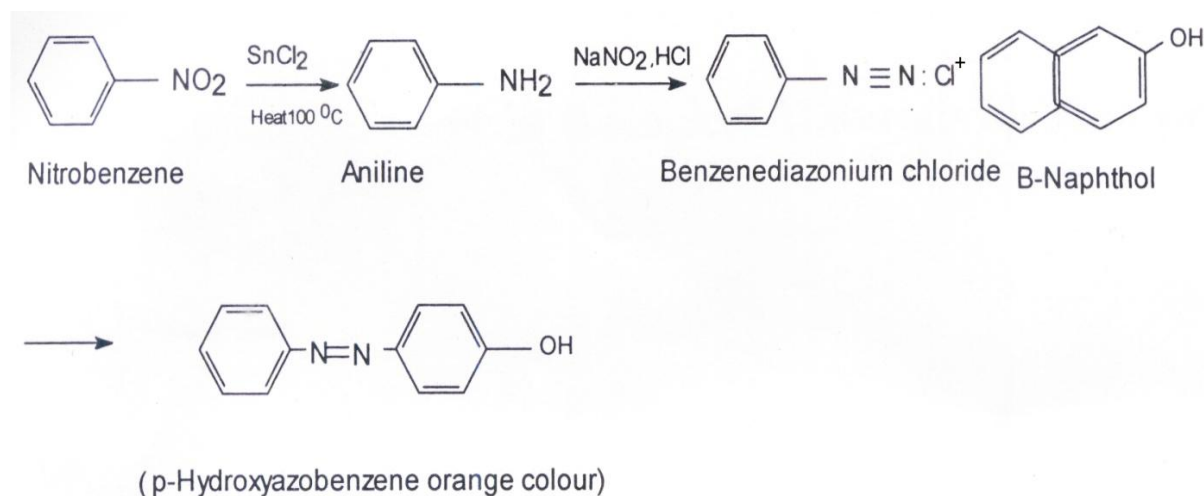


Figure 1

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